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# BACTERIAL GROWTH IN TRAY PACK ACIDIFIED RICE

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Acidification of white rice was shown to be ineffective in preventing growth of sporeforming bacillus species. Moreover, there was nonuniform distribution of the acidulant, which resulted in portions of the acidified rice that were less acidic. It was concluded that the high bacterial counts encountered in this product were not due to contamination or improper processing. Rather, they were due to growth of heat-resistant, sporeforming bacteria found naturally in raw rice, notably, Bacillus coagulans, which were able to survive the pasteurization processing temperature.

Because of the potential for spoilage that was indicated by this investigation, Tray Pack acidified white rice was replaced by Spanish Rice and a nonacidified thermally processed white rice.

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# PREFACE

Tray Pack (half size, metal, steamtable trays) food products are an integral component of the United States Army Combat Field Feeding System. To ensure that all Tray Pack (TP) items meet shelf life requirements, storage studies are conducted at temperatures encountered in the field. Microbiological testing for commercial sterility before, during, or after storage is performed to help assure that TP products are properly processed, stored and distributed safely and without spoilage.

Routine testing of TP acidified white rice at the U.S. Army Natick Research, Development and Engineering Center (Natick) demonstrated that this product, which was produced by two manufacturers, was not commercially sterile. The concern for potential spoilage prompted the following study. The purpose of this study was to determine if the high bacterial counts encountered were due to contamination, to improper processing, or to growth in the product during storage.

This study was performed by the Microbiology Branch, BioScience Division, Science and Advanced Technology Directorate, Natick Research Development and Engineering Center, Natick, Massachusetts. The principal investigator was Edmund M. Powers.

The authors wish to acknowledge the assistance of James Halkiotis of the Food Engineering Directorate at Natick for providing the TP rice.

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## INTRODUCTION

Tray Pack (TP) acidified white rice was developed as a military ration to fulfill the need for a starch component that could be served with a variety of entree items. Attempts have been made in the past to obtain shelf-stable precooked rice.[1, 2] However, thermal processing at 100°C (212°F) caused excessive starch leaching, cohesiveness, discoloration, matting, loss of kernel integrity and poor cooking qualities. To produce an acceptable and commercially sterile product by retorting at 100°C, it was necessary to adjust the pH of the rice below 4.6,[2] for the purpose of inhibiting the spore forming bacteria and preventing the growth of Clostridium botulinum.

Among a series of acidulants in the Generally Regarded As Safe (GRAS) list of the United States Food and Drug Administration, glucono-delta-lactone was selected for the rice because it imparted less tartness than other acids.

This study was initiated following an observation during routine analysis that TP acidified rice samples exhibited high levels of bacteria (aerobic plate count of $10^3$ to $10^5$ per gram) after incubation at 25°C for 10 days. The objective was to determine whether acidification was sufficient to prevent bacterial growth in rice.

## MATERIALS AND METHODS

### Rice Samples

Rice used in this study was obtained from TP acidified white rice (Federal Specification N-R-002189) produced by two different manufacturers.

Rice from one TP source stored at 4°C (40°F) was used for the inoculation studies carried out in Thermal Death Time (TDT) cans. The TDT cans were filled with 16 grams of the acidified rice (pH 4.2) and inoculated with a mixed microflora made up of six cultures, which were previously isolated from TP acidified rice during routine analysis and are described below. Each can was sealed under a vacuum of 10 inches of mercury and incubated. Uninoculated rice samples served as controls.

Tray Pack acidified rice from another manufacturer was used to measure the growth of indigenous microflora (the rice was not inoculated). This rice, in an unopened TP, was stored in the laboratory for seven days and then refrigerated until filled into plastic Whirlpak bags (NASCO). Three Whirlpak bags were each filled with 200 grams of the acidified rice (pH 4.1) and closed; one bag was incubated at each temperature.

## Inoculum

A mixed inoculum consisting of Bacillus sphaericus, Bacillus circulans and four strains of Bacillus coagulans was prepared. All cultures had been previously isolated from TP acidified rice during routine analysis and were identified by conventional procedures.[3] For the inoculum, each culture was grown in trypticase soy broth supplemented with 0.1 percent yeast extract at 35°C (95°F), for 24 hours. The inoculum was prepared by adding one mL of each culture to a sterile test tube and vortexing. The mixed culture was diluted 1:1000, in Butterfield's[4] sterile buffered water (SBW), and 0.1 mL was added to each TDT can containing acidified rice. All operations were carried out in a vertical laminar flow bench.

## Incubation of the Rice

Sealed and evacuated TDT cans containing inoculated and uninoculated acidified (pH 4.2) rice were incubated at 25°C, 35°C, and 55°C (77°, 95°F, and 131°F) for five days. Two cans were removed from each temperature for bacterial analysis.

Uninoculated acidified (pH 4.1) rice filled into Whirlpak bags was incubated at 25°C, 35°C, and 55°C for 14 days. Aerobic plate counts were performed at 0, 3, 5, 7, and 14 days.

Bacterial Enumeration

Standard aerobic plate count (APC) procedures[4] were performed to measure bacterial growth in the rice. The APC's were performed on inoculated and uninoculated acidified rice, in TDT cans, by removing duplicate cans of rice from each storage temperature after two and five days. The entire contents of each can were weighed into a sterile blender jar, diluted 1:10 with SBW and blended for two minutes. Tenfold dilutions were prepared in 90 mL of SBW. One mL of each dilution was pipetted into duplicate petri plates and poured with plate count agar (PCA). Plates were incubated for 72 to 96 hours at the same temperature at which the rice samples were stored. For example, if the rice was stored at 25°C, the APC was performed at 25°C. However, initial APC's were obtained from plates incubated at 35°C, immediately after the rice was inoculated.

The indigenous microflora in the acidified rice (pH 4.1) contained in Whirlpak bags were enumerated over a 14-day period by the same APC procedure described above. At each sampling interval 10 grams of rice were aseptically removed from each bag stored at 25°C, 35°C, and 55°C. The rice was blended in 90 mL of SBW. Additional tenfold dilutions were prepared, plated into duplicate petri plates and mixed with PCA as described above. The petri plates were incubated for 72 to 96 hours at the same temperature at which the rice samples were stored; in the same manner as described above for rice stored in TDT cans.

Measurement of pH. The pH of the rice was measured using a Beckman pH meter (model SS-2) employing a combination electrode.

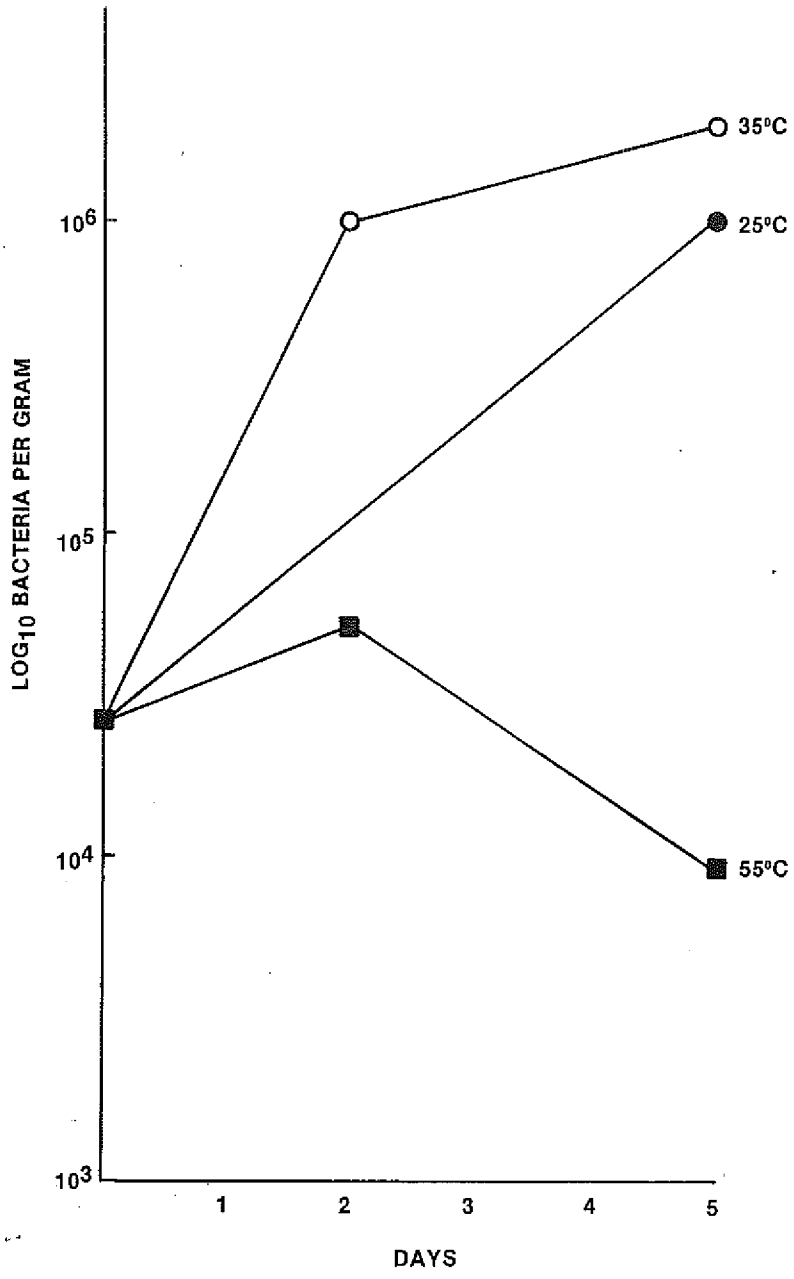


Figure 1. Bacterial growth in canned (TDT) inoculated acidified rice at 25°C, 35°C, and 55°C.

Measurement of vacuum. The vacuum in each can tested was measured with a Budenberg vacuum gauge. Readings were in inches of mercury.

## RESULTS

Figure 1 depicts the growth of bacteria inoculated into acidified (pH 4.2) rice contained in TDT cans. Initial counts indicated that each inoculated can received an inoculum of $3.2 \times 10^4$ mixed bacteria per gram of rice. The APC of uninoculated rice was less than 10 per gram in three replicate samples.

At 35°C the bacteria grew rapidly, reaching $1.2 \times 10^6$ per gram in only two days and doubling to $2.4 \times 10^6$ per gram after five days. Although bacterial growth at 25°C was slower, the APC at this temperature reached $1.2 \times 10^6$ per gram after five days. At 55°C after two days, the bacterial count approximately doubled, reaching $6.1 \times 10^4$ per gram and then declined to $1 \times 10^4$ per gram after five days. Rapid decline of bacteria and autosterilization in an acid product at 55°C is not unusual. Since growth was clearly evident at all three temperatures, the experiment was terminated after five days.

Table 1 shows the vacuum (gage pressure) in the TDT cans filled with inoculated rice and the pH of the rice during storage. The pH and vacuum were the same for the uninoculated rice and are not presented. The slight variation in pH was considered to be within experimental error and the

sensitivity of the pH meter. A vacuum was still measurable after 2 to 5 days at all three storage temperatures. This fact indicated that the growth depicted in Fig. 1 took place under anaerobic conditions.

Table 1. Vacuum and pH of Inoculated Acidified Rice in TDT Cans During Storage

| | Storage Temperature | | | | | |
|---|---|---|---|---|---|---|
| | 25° | | 35° | | 55° | |
| Days | Gage Pressure* | pH | Gage Pressure* | pH | Gage Pressure* | pH |
| 0 | 10 | 4.2 | 10 | 4.2 | 10 | 4.2 |
| 2 | 2 | ND | 2 | ND | 2 | ND |
| | 2 | | 3 | | 0 | |
| 5 | 1 | ND | 2 | ND | 3 | ND |
| | 0 | | 2 | | 0 | |
| 9 | 3 | 4.2 | 0 | 4.0 | 0 | 4.3 |
| | 2 | 4.1 | 2 | 4.0 | 0 | 4.3 |

* Gage Pressure - Vacuum in inches of Mercury
ND-Not done

Figure 2 shows the aerobic growth of indigenous bacteria in uninoculated acidified rice (pH 4.1) contained in Whirlpak plastic bags. The rice in an unopened Tray Pack had been stored at ambient temperature (23° to 25°C--73° to 77°F) for seven days and then refrigerated before it was filled into the Whirlpak bags. Initial counts showed that the starting bacterial population in the rice was $2.4 \times 10^4$ per gram. This count was typical of acidified rice in Tray Packs routinely incubated at 25°C, and probably represented growth in the rice. The indigenous microflora continued

to increase during aerobic incubation in Whirlpak bags, reaching $10^7$ per gram after five and seven days at 35°C and 25°C, respectively. No growth occurred in the rice incubated at 55°C, indicating that thermophiles were either not viable or were not present in this rice.

## DISCUSSION

This study demonstrated that TP acidified white rice did not effectively inhibit growth of sporeforming Bacillus species, which were naturally present in the rice, or inoculated into it. The bacteria grew under partial vacuum as well as aerobic conditions at 25°C and 35°C. Growth also occurred at 55°C, under partial vacuum, due undoubtedly to the presence of B. coagulans, a facultative thermophile, which was inoculated into the rice. The rice was acidified with glucono-delta-lactone (GDL) because, unlike other acidulants, it did not impart a "sharp" flavor.

The uncooked rice had spore counts ranging from 35 to 95 per gram and included at least three sporeforming bacterial species, which were recovered from the acidified, thermally processed product (heated to an internal temperature of 92°C (195°F). Two of the three species, including B. coagulans, were facultative anaerobes capable of growing in the rice under both anaerobic and aerobic conditions (Figs. 1 and 2). The recovery of B. coagulans was particularly significant because this sporeforming microorganism has considerable heat resistance and is known to cause economic, flat-sour spoilage in canned products in the pH range of 4.1 to 5.0.[4] Since the pH range specified for TP acidified rice is only 4.3 to 4.6 (Federal

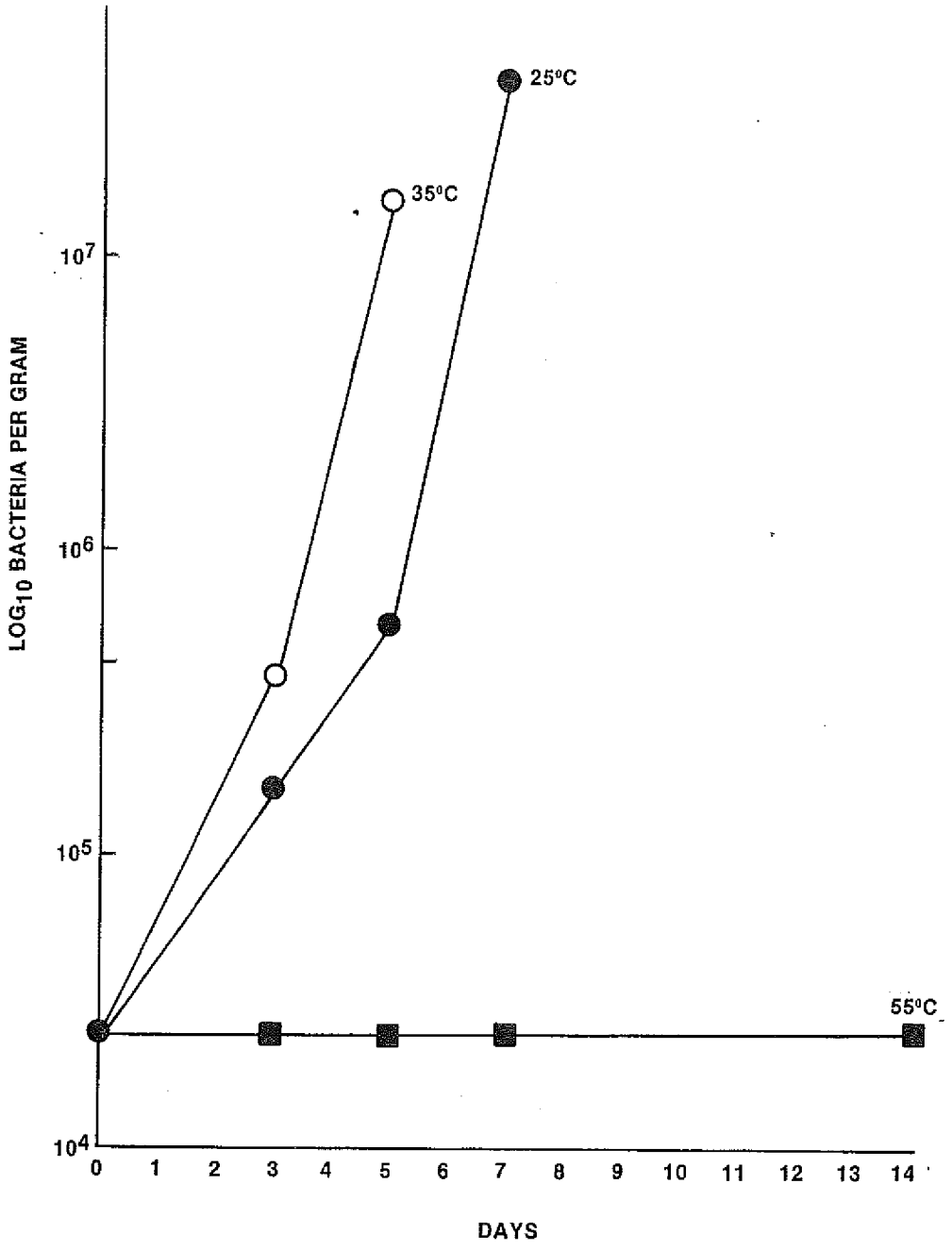


Figure 2. Growth of indigenous microflora in acidified (pH 4.1) rice packaged in Whirlpak bags at 25°C, 35°C, and 55°C.

Specification N-R-002189), growth of B. coagulans may not be inhibited on the basis of pH alone.

It was concluded that the high bacterial counts encountered in this product were not due to contamination or improper processing. Rather, they were due to growth of heat-resistant sporeforming bacteria found naturally on raw rice, notably B. coagulans, which were able to survive the pasteurization processing temperature.

The equilibrium pH of the TP rice was not well established as demonstrated by SATD's Physical Science Division (Personal communication, Jarboe, 1985). The pH in that study varied between 4.2 and 4.6 not only between TPs, but also within a TP. It is possible, considering the variability displayed, that pockets of even higher pH existed, which may have allowed repair of injured spores and unrestricted growth.

It is recommended that effects of acidulants on the growth of bacteria be investigated since some acidulants have greater inhibitory and lethal properties on the basis of pH and heat than do others. More research is also required to develop an effective heat process for acidified white rice as well as reliable methods for establishing uniform distribution of the acidulant.

Because of the potential for spoilage that was indicated by this investigation, TP acidified white rice has been withdrawn as a military ration. It has recently been replaced by two other TP rice products, Spanish rice and nonacidified white rice. Both products are commercially

sterilized by heat processing. Spanish rice is heated at 200°F to 220°F (93.3°C to 104°C) for 30 minutes. The pH ranges from 4.1 to 4.4. Investigations are underway to improve the texture of white rice when retorted to an $F_o$=6. Two possible solutions include: (1) reduction of the $F_o$ combined with pH control and (2) pasteurization at 200°F with total pH control.

This document reports research undertaken at the US Army Natick Research and Development Command and has been assigned No. NATICK/TR-88/006 in the series of reports approved for publication.

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